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Original Article

EVALUATION OF THE EFFECT OF DICLOFENAC SODIUM ON INH-INDUCED SEIZURES

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ABSTRACT

Epilepsy is a common and a complex neurological disorder that is characterized by a persisting tendency to generate seizures which affect quality of life. However neuro inflammation may be included as a major contributor to seizure that may be treated via non-steroidal anti-inflammatory drugs.

Aims: To evaluatie diclofenac sodium's effect on seizure activity and to assess its effect on seizure, if combined with diazepam.

Material and methods: Twenty mice were randomly allocated to four groups (five mice each) where the first group was served as negative control group, given distilled water, second group served as positive control group, given diazepam (2mg / kg, i.p), third group served as tested group and received diclofenac sodium (10 mg /kg, IP) and the last group served as another tested group and received combination of diclofenac sodium (10 mg / kg, i.p) plus diazepam (2 mg / kg, i.p). Isoniazid (convulsion inducer) had been injected intraperitoneally 30 minutes after drug administration. The results: the effect of pre-administration of diclofenac sodium intraperitoneally at a dose of 10 mg/kg on the onset of action was highly significant in comparison with first group p-value < 0.001 and was significant and strongly significant in comparison with both diazepam and the combination groups (p-value = 0.003 and 0.001 respectively). The time recorded as a seizure duration which belong to the effect of diclofenac sodium was 57.4 ± 8.142 seconds with highly significant effect against all remaining groups. However, it was revealed that no death was happening in diclofenac sodium group, but it reaches to 100% in diazepam group while in group of combined treatment it was 60% through 24 hours that followed the study period.

Conclusion: Diclofenac sodium (non-selective NSAIDs) may display protection against seizures, but this protection is revealed to be a lesser extent when it combined with diazepam.

KEYWORDS: Anticonvulsant Effect, Diazepam, Diclofenac Sodium, Mice, INH & Seizure

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INTRODUCTION

Epilepsy is a debilitating neurological disorder in humans characterized by spontaneous and recurrent seizure episodes[1].It forces a tremendous burden on patients and their families in addition to their society, which making epilepsy as a costly disorder. Moreover, the estimates about people who have epilepsy are that more than 65 million people worldwide [2].

In spite of the vast number of drugs introduced for the treatment of epilepsy, but the need for agents with some properties such as good bioavailability, less side effects, low cost, and rapid onset of action is urgent.

Several recent studies have highlighted the inflammation's role in the changes that participate to seizures and the occurrence of epilepsy .Inflammatory changes reported in epilepsy involve microglia activation and damage of blood brain barrier in combination with an increamediator expression, including neurotoxic mediators expression including cytokines, chemokines, arachidonic acid in a them have directly contributed several of the mare [directlycontributed to neuronal injury[6-9]

Interestingly, the cyclooxygenases (COX) are the key enzymes that convert arachidonic acid to prostaglandins which have been engagedto physiological and pathophysiological functions in the central nervous system [10]. In respect to the correlation between cyclooxygenase enzymes and the pathophysiology of epilepsy, some studies highly suggest the use of COX-inhibitors as an adjuvant therapy in the treatment of epilepsy.[10-12]

Generally, non-steroidal anti-inflammatory drugs NSAIDs, inhibitors of cyclooxygenase enzymes, are used to provide analgesic, antipyretic, and anti-inflammatory effects. However, the role of some individuals of NSAIDs in the seizure activity is positive and the others have negative effect [13].

Diclofenac sodium, an NSAID, inhibitsCOX enzyme and effect on arachidonic acid release and uptake. Clinically, diclofenac sodium is employed in pain relief, fever, and also in different inflammatory conditions such as acute gout, osteoarthritis and rheumatoid arthritis, dysmenorrhea, bursitis, ankylosing spondylitis. Furthermore,it is used in some postoperative inflammatory conditions[14].

Interestingly, the effect of diclofenac sodium alone on seizures has beenstudied only twice, one of them accomplished in Libyain 2014 [15] which concluded that 10-20 mg/ kg of diclofenac sodium reduced onset time in comparison with the diazepam treated group in picotoxin (PTX) induced seizureswhile the other study achieved in Brasilin 2016[16]which concluded that diclofenac sodium with different doses of 5-10mg/kg decreased severity of seizures in the pentylenetetrazolePTZ induced seizures.

Besides that, the previous study recommended that a new therapeutic way is needed in the treatment of epilepsy with diclofenac sodium. With this background, the present study was aimed to investigate the effect of diclofenac sodium on seizure susceptibility following INH-induced seizures in mice and to evaluate its effects on the anticonvulsant activity of diazepam when they are given together.

MATERIALS AND METHODS

Animals

Locally bred adult male mice weighing (20-32) g were purchased from Thi-Qar University/College of Sciences/Biology department. All mice were maintained on a 12-hour (light: dark) cycle and were allowed for free access to food and water. However, mice were acclimated to the testing room for at least 30 minute sprior starting the test.

Chemicals and solutions

Diclofenac sodium, diazepam, isoniazid (INH), and distilled water were used in this study.

Experimental Design

Experiments were performed on twenty mice,. They were divided into four groups randomly (five in each) as the following:

- **1st Group:** Negative control group received distilled water.
- **2nd Group:** Positive control group received diazepam (2mg/kg i.p)
- **3rd Group:** Tested group received diclofenac sodium (10 mg/kg i.p)
- **4th Group:** Another tested group received diclofenac sodium (10 mg/kg i.p) + diazepam (2 mg/kg i.p)

Thirty minutes after treatment, 200 mg/kg of a freshly prepared solution of INH (a chemical induced seizures) was administered to each mouse, as intraperitoneal inj.

Immediately after INH administration, the mice were individually placed under the glass funnels and observed for 90 minutes for the following parameters:

Onset time of seizures in seconds, duration of seizures for each episode, and percentage of death[17, 18].

The anticonvulsant activity for tested drug was reflected by prolongation of the seizures onset time, reduction in the duration of seizures[17]or by its protection against death. The mortality rate could be observed for twenty four hours after INH administration.

Statistical Analysis

Data were analyzed using SPSS version 19. The results were expressed as mean ± SD. The significance of the difference in the responses of the treatment groups in comparison with the each other was determined by one-way analysis of variance (ANOVA) followed by $P < 0.05$ was considered statistically significant while $P < 0.001$ was considered statistically highly significant.

RESULTS

The investigation of a time dependent effect through a period of this study had been followed. Table 1 explained the effect of each of distilled water, diclofenac sodium, diazepam, and the combination (diclofenac sodium plus diazepam) on the onset time in INH induced seizure in mice. Administration of 200 mg/kg of isoniazid (INH) to mice was found to induce seizure at a mean of time of 71 ± 15.716 seconds in a negative control group while in a positive control group the onset mean was 194.2 ± 4.438 seconds and the effect of diazepam was highly significant (p-value < 0.001) in a comparison with the negative control group. Further, the effect of pre-administration of diclofenac sodium intraperitoneally in a dose of 10 mg/kg on the onset of action was also highly significant in comparison with first group p-value < 0.001 and was significant and strongly significant in comparison with both diazepam and the combination groups (p-value = 0.003 and 0.001 respectively).Moreover, the intraperitoneal pre-administration of combined agents (diclofenac sodium plus diazepam) was significantly prolonged onset time 202.8 ± 3.493 seconds (p <0.001) against negative control group and diclofenac sodium group while it was insignificant when it compared with a positive control group (p-value = 0.831).

Table 1: Effect of Drugs on onset of Seizures in INH Induced Seizures in Mice

Groups(N=5)	Treatment Groups	Onset of Seizure (Sec.)
G1	Distilled water + INH	71 ± 15.716
G2	Diazepam + INH	194.2 ± 4.438*

Table 1: Contd.,		
G3	**Diclofenac sodium+ INH**	**151.2 ± 27.399***
G4	**Diclofenac sodium + Diazepam + INH**	**202.8 ± 3.493****
***Statistically significant against negative control group **Statistically significant against diclofenac sodium group**		

Moving to the effect of the drugs on duration of seizure (table 2), the time recorded as a seizure duration which belong to the effect of diclofenac sodium was 57.4 ± 8.142 seconds with highly significant effect against all remaining groups. However, the effect of combination on the duration of seizure was highly significant against both negative control group and diclofenac group (p-value < 0.001), but it was insignificant against diazepam group (p-value = 0.421).

Table 2: Effect of Drugs on Duration of Seizures in INH Induced Seizures in Mice

Groups (N=5)	Treatment (Groups)	Duration of Seizures (Sec.)
G1	**Distilled water + INH**	**133.6 ± 12.44**
G2	**Diazepam + INH**	**20.8 ± 4.26***
G3	**Diclofenac sodium+ INH**	**57.4 ± 8.14***
G4	**Diclofenac sodium + Diazepam + INH**	**13 ± 2.64****
***Statistically Significant Against Negative Control Group **Statistically Significant Against Diclofenac Sodium Group**		

Percent of mice alive in the present study had been calculated and recorded in figure 1. It was revealed that no death was happening in all groups at the end of the study period (90 minutes). However, the percent of alive mice in diazepam group were 40%, while in group of combined treatment, it was zero percent through 24 hours that followed the study period.

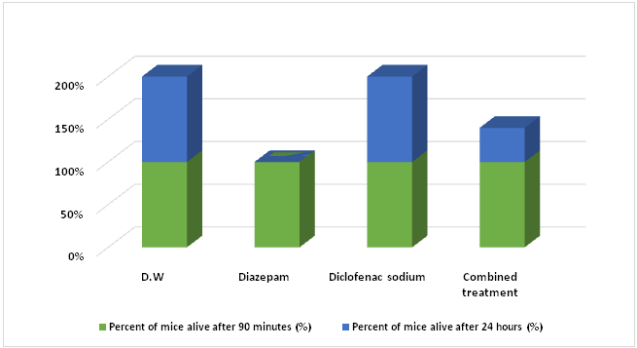

Figure 1: Percent of Mice Alive After Two Time Periods

DISCUSSIONS

Epilepsy disorder impacts millions of persons world-wide [19] at a time many patients became refractory to antiepileptic medications that paid us to urgently investigate new agents. Notably, brain inflammation role in epilepsy has been reported in repeated manner recently[7]. Hence, drugs that have anti-inflammatory potential such as diclofenac sodium may provide rapeutically way in reducing epilepsy.

From our findings that was explained in the result section, the distilled water given group that followed by INH administration revealed that the onset of seizure has been repaid in addition to longer duration of seizure among other studied groups. Isoniazid (isonicotinic acid hydrazide) INH(a GABA-synthesis inhibitor) has been used in this study to produce the animal model of chemically induced seizure because this model is available in our country, Iraq, and it is proven to be of value for evaluation drugs that may have anti-convulsive effect[20]. All mice in this group were alive.

Another control group in this study is diazepam group "positive control group". It is anti-epileptic agent acts by binding to GABA receptors. Our results have been reported as it is proved that diazepam can protect mice from seizures induced by INH and this was clear from its positive effect on both of the onset and duration of seizures. In contrast to previous study [15], the death percent in this group was 100% that may be analyzed by the effect of environmental condition at that time on mice where the experimental room environment was hot, making mice intolerant and resulting in their death through 24 hours period.

On the other hand, inflammatory process plays a pivotal role in some diseases such as epilepsy [21, 22] where there is COX enzyme up-regulation following seizure activity. Moreover, it is documented that prostaglandins are found in the brain [23] and their levels are raised through their release from glial cells after seizure induction in animal models[24]. In agreement with previous study that was published by Hind G Almaghour*et al*, 2014, our study demonstrated that diclofenac sodium delayed seizure onset time with maintaining all mice alive along study period, but the percentage of mice alive is inconsistent with that of previous mentioned study where the death percentage was 25% that may be translated by the effect of type of convulsive agent used. Notably, seizure duration in this trial has been reduced in comparison to that of negative control group which gives reflection to the protective effect of this NSAID from convulsions.

In similarity with the findings of previous study in 2014 [15] that was achieved in Libya, present outcomes have revealed that following pretreatment with combined agents(diazepam and diclofenac sodium)a much stronger protective effect against isoniazid-induced seizures in comparison to diclofenac sodium alone, but this effect was statistically insignificant when compared with that of diazepam alone. Death percent was recoded as 60% that may belong also to the environmental conditions at that time.

CONCLUSIONS

Based on this study results, using diclofenac sodium in the INH induced seizures, provided protection from seizures in animals treated with doses of 10 mg/kg, while it's gathering with diazepam gave point to be similar to the neuroprotective effect of diazepam when administered alone. Absolutely, additional studies are needed to demonstrate the advantages of diclofenac sodium and its combination with diazepam in epilepsy and to evaluate their effect on mortality rate in a place with a better environmental conditions.

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